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(54) Title: MYCOBACTERIUM AS ADJUVANT FOR ANTIGENS

(57) Abstract

Immunoregulatory material from a mycobacterium other than *M. tuberculosis*, especially killed cells of *M. vaccae*, is an advantageous adjuvant for administration with antigens (including allergens).

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+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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Mycobacterium as adjuvant for antigens

The present invention relates to carriers, and more particularly adjuvants, for antigens (including allergens), for use in vaccination, and other ways of altering, in a favorable way, the immune response to an antigen.

Killed cells of M. vaccae are known to be useful as immunotherapeutic agents in mycobacterial diseases such as tuberculosis and leprosy (see GB-A-2156673). This known use of M. vaccae may rely upon the stimulation of T-cell mediated immunity to endogenous antigens of M. vaccae. Killed cells of M. vaccae are also useful in the treatment of various autoimmune diseases including rheumatoid arthritis, ankylosing spondylitis and Reiter's syndrome (see PCT/GB 85/00183).

The present invention is founded upon the surprising observation that killed cells of M. vaccae can be used to stimulate and/or modify in a favorable way the immune response to antigens which are not endogenous to M. vaccae .

The immune response to an antigen has two distinct aspects: (1) selection of an epitope (antigen fragment) as an initiator of, and target for, the response; and (2) selection of a particular immune response mechanism as the response directed against the particular epitope selected. Current methods of stimulating the immune response, e.g.

vaccination, have generally concentrated on the first aspect, but it has become clear, in the light of recent research, that it is essential that the immune response shall be stimulated or modified to a favorable way, since it is possible to modify the immune response unfavorably, leading for example to increased susceptibility to infection. One of the surprisingly beneficial properties of killed cells of M. vaccae in that they promote selection of a favorable immune response mechanism.

It is known that, at least in the mouse (see, e.g. Mosmann & Moore, Immunology Today, 1991, A49-A53), different T-cell subsets have different patterns of cytokine secretion. T_H2 cells express interleukin(IL)-4, IL-5 and IL-10, whereas T_H1 cells produce IL-2, γ-interferon (IFN-γ) and lymphotoxin. The T_H2 cells are involved in the pattern of immune responses seen in, e.g., asthma, pollen allergies, and eczema, while T_H1 cells are involved in the pattern used in killing intracellular parasites. It appears that killed cells of M. vaccae promote the immune response characteristic of T_H1 cells.

Conversion of the T cell component of the response to allergens from the T_H2 pattern to the T_H1 pattern reduces or terminates symptoms of conditions such as asthma, hay fever, and atopic eczema, by reducing production of IgE, reducing recruitment of eosinophils and

mast cells to the inflamed site, and greatly increasing the antigen concentration required to trigger a response (because the T_H1 response requires a much higher concentration of antigen to be triggered than the T_H2 response). Consequently the levels of allergen in the environment become insufficient to trigger symptoms.

It also appears that killed cells of M. vaccae may promote the immune response characteristic of T_H1 cells, and in the case of autoantigens, enhance reduction of the response via the immunoregulatory network.

The beneficial effect of using killed M. vaccae as an adjuvant may also be associated with the 65 kDa mycobacterial heat shock protein (hsp 65) described by Young et al. "Stress proteins are immune targets in leprosy and tuberculosis", Proc. Natl. Acad. Sci. U.S.A. 85 (1988), pp4267-4270 in form obtained from M. bovis. The preferred autoclaved M. vaccae cells used in the present invention as described below are believed to provide an effective package of adjuvant, hsp 65 and other substances.

The immunoregulatory material derived from M. vaccae or another mycobacterium other than M. tuberculosis may be administered with or separately from the antigen exogenous to the mycobacterium to achieve an improved response to the antigen.

M. tuberculosis is the causative agent of tuberculosis and an avirulent variant of it is used in the

production of the BCG used vaccine against tuberculosis in immunization programmes throughout the world. Immunoregulatory material from M. tuberculosis should not be used in accordance with the present invention in order to avoid compromising the use of BCG vaccine by inducing tuberculin test positivity or reducing the subsequent efficacy of BCG. For these reasons the use of immunoregulatory material from M. tuberculosis is excluded from the present invention.

It is believed that material from mycobacterial species other than M. tuberculosis might be useful in accordance with the present invention. However, especially as it is already a known immunotherapeutic agent, immunoregulatory material from M. vaccae is currently preferred.

The invention accordingly provides a product comprising immunoregulatory material derived from a mycobacterium other than M. tuberculosis and an antigen exogenous to the mycobacterium as a combined preparation for simultaneous, separate or sequential use for promoting T cell-mediated response to said antigen.

The product of the invention conveniently, and therefore preferably, comprises dead cells of M. vaccae, most preferably cells which have been killed by autoclaving or by irradiation. The product normally comprises more than 10^8 microorganisms per ml of diluent, and preferably

from 10^8 to 10^{11} killed M. vaccae microorganisms per ml of diluent.

The diluent may be pyrogen-free saline for injection alone, or a borate buffer of pH 8.0. The diluent should be sterile. A suitable borate buffer is:

$Na_2B_4O_7.10H_2O$	3.63 g
H_3BO_3	5.25 g
NaCl	6.19 g
Tween 80	0.0005%
Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.D. Paul, Ann. Soc. Belge Med, Trop. 1973, 53 141-389). The strain is a stable rough variant and belongs to the aurum sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Juhasz., Zentr albl. Bakteriol. Parasitenkd. Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133).

The strain denoted R877R has been deposited under the Budapest Convention at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

For the preparation of the product of the invention, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immunol, 1955 75, 15) solidified with agar. Preferably the solid medium contains 1.3% agar. The medium inoculated with the microorganisms is incubated aerobically to enable growth of the microorganisms to take place, generally at 32°C for 10 days. The organisms are harvested, then weighed and suspended in a diluent. The diluent may be unbuffered saline but is preferably borate-buffered and contains a surfactant such as Tween 80 as described above. The suspension is diluted to give 200 mg of microorganism/ml. For further dilution, borate buffered saline is preferably used so that the suspension contains 10 mg wet weight of microorganisms/ml of diluent. The suspension may then be dispensed into suitable multidose vials (e.g. 1 ml). Although the microorganisms in the vials may be killed using irradiation, e.g. from ^{60}Cobalt at a dose of 2.5 megarads, or by any other means, for example chemically, it is preferred to kill the microorganisms by autoclaving, for example at 10-15 psig (69-104 kPa) for 10-15 minutes (115°-125°C). It has been discovered, unexpectedly, that autoclaving yields a more effective preparation than irradiation.

Extracts or fractioned portions of the

microorganisms can also be used provided, of course, they have the required adjuvant effect.

The immunotherapeutic product of the invention comprises an association of an effective, non-toxic immunomodifying amount of an immunoregulatory material from a mycobacterium other than M. tuberculosis, especially M. vaccae, and of an effective, non-toxic, immunity-stimulating amount of an antigen exogenous to the mycobacterium.

The exogenous antigen may be any antigen against which it is desired to stimulate T-cell mediated immunity or to alter the nature of the T-cell response, to achieve palliation or cure of the infection or other condition to be treated. Examples include antigens associated with diseases at present regarded as having an autoimmune aetiology such as multiple sclerosis, antigens associated with chronic viral infections such as hepatitis, bovine spongiform encephalopathy (BSE), and myoencephalitis (ME), antigens associated with cryptic parasite infections such as leishmaniasis and trypanosomiasis, and allergens (e.g. those present in pollens, animal dander, and house dust mite) responsible for such conditions as hayfever, asthma, food allergy and eczema. The immunotherapeutic product of the invention incorporating the appropriate exogenous antigen may be used prophylactically or therapeutically.

The exogenous antigen may be produced by any

conventional technique, such as by culture and killing or attenuating the disease organism to provide a killed or attenuated vaccine, by separation and purification of the antigen, with optional chemical modification thereof, from a disease organism or, in the case of proteinaceous antigens, by expression of a gene encoding the antigenic protein in a suitable recombinant organism.

The exogenous antigen may be combined with the immunoregulatory mycobacterial material by admixture, chemical conjugation or adsorption using conventional techniques. Alternatively the exogenous antigen may be produced by expression of an exogenous gene (for instance contained within a plasmid, cosmid, viral or other expression vector or inserted into the genome of the mycobacteria) in the mycobacteria from which the immunoregulatory material is also produced. Thus, for instance, recombinant M. vaccae may be cultured so as to achieve expression of the exogenous antigen and then killed and processed as described above, or under such conditions appropriately modified to preserve the biological activity of the exogenous antigen, to provide an immunoregulatory material containing the exogenous antigen. Techniques for obtaining and expressing such exogenous genes are conventional.

The therapeutic agent is in general administered by injection in a volume in the range 0.1-0.2 ml, preferably

0.1 ml, given intradermally. A single dosage will in general contain from 10^7 to 10^{10} killed M. vaccae microorganisms. It is preferred to administer to patients a single dose containing 10^8 to $2x10^9$ killed M. vaccae. However, the dose may be repeated depending on the condition of the patient.

The amount of exogenous antigen administered in association with the M. vaccae is in general the same amount as has previously been used when the given antigen has been administered to provide an immune response. In the case of antigens involved in hay fever and asthma, the required dosage depends on the manner in which the antigen is extracted and specific dosages which are generally applicable cannot be given, although therapeutic preparations containing such antigens are well known, see the article on "Desensitising vaccines", Brit. Med. J. 293 (1986) p.948. For other types of antigen not involved in hay fever or asthma, the usual dosage is in the range of 0.1 to 5 μg.

The therapeutic agent may be administered with the antigen, typically in admixture, but it is within the scope of the invention to administer, e.g. by injection, first the therapeutic agent, e.g. killed cells of M. vaccae, and then, into the same site, the exogenous antigen.

Although the therapeutic agent will generally be administered by intradermal injection, other routes, e.g.

oral administration, can also be used.

The invention includes within its scope a method of treatment of the human or animal body which comprises administering an effective non-toxic amount of immunoregulatory material derived from a mycobacterium other than M. tuberculosis and, with or following the said material, of an antigen exogenous to the mycobacterium to a human or animal in need of T-cell mediated immunity against the exogenous antigen or otherwise in need of the pattern of T-cell mediated response against the exogenous antigen promoted by the said immunoregulatory material.

The invention further provides the use, in the manufacture of an immunotherapeutic composition for use in treatment of the human or animal body by promoting the T-cell mediated response to an exogenous antigen, of immunoregulatory material derived from a mycobacterium other than M. tuberculosis, and pharmaceutical formulations comprising an association of the said immunoregulatory material and an antigen exogenous to the mycobacterium and one or more diluents or carriers therefor.

The pharmaceutical formulation can contain further ingredients such as additional adjuvants, preservatives, stabilisers etc. It may be supplied in sterile injectable liquid form or in sterile freeze-fried form which is reconstituted prior to use.

The following Example illustrates the invention.

EXAMPLE

M. vaccae NCTC 11659 is grown on a solid medium comprising modified Sauton's medium solidified with 1.3% agar. The medium is inoculated with the microorganism and incubated for 10 days at 32°C to enable growth of the microorganism to take place. The microorganisms are then harvested by gently scraping the surface of the agar and weighed (without drying) and suspended in M/15 borate buffered saline at pH8 to give 10 mg of microorganisms/ml of saline. The suspension is dispensed into 5 ml vials, and then autoclaved for 15 minutes at 15 psi (104 kPa) and about 120°C to kill the microorganisms. This is then dispensed into suitable multidose vials. After cooling, 1/10th volume of exogenous antigen (at the standard concentration of 2µg/ml) is added. The therapeutic agent thus produced is stored at 4°C before use. A single dose consists of 0.1 ml of the suspension, which should be shaken vigorously immediately before use, containing 1 mg wet weight of M. vaccae and 0.02 µg of exogenous antigen. The dose is given by intradermal injection normally over the left deltoid muscle.

Only one dose is normally required. The patient should not receive high dose steroids or other immuno-suppressive therapy. Up to six months may elapse before the beneficial effect becomes apparent.

CLAIMS

1. A product comprising immunoregulatory material derived from a mycobacterium other than M. tuberculosis and an antigen exogenous to the mycobacterium as a combined preparation for simultaneous, separate or sequential use for promoting T cell-mediated response to said antigen.

2. A product according to claim 1, wherein the immunoregulatory material derived from a mycobacterium comprises dead cells of M. vaccae.

3. A product according to claim 2, wherein the cells of M. vaccae have been killed by autoclaving.

4. A product according to claim 2 or claim 3, wherein the immunoregulatory material derived from M. vaccae comprises the 65 kDa heat shock protein.

5. A product according to any one of claims 2 to 4 wherein the material derived from M. vaccae is derived from the strain as deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

6. A product according to any one of claims 1 to 5 comprising per dose, immunoregulatory material from 10^7 to 10^{10} M. vaccae microorganisms.

7. A method of treatment comprising administering an effective non-toxic amount of immunoregulatory material derived from a mycobacterium other than M. tuberculosis and

of an antigen exogenous to the mycobacterium, to a human or animal in need of T-cell mediated immunity against the exogenous antigen, or otherwise in need of the pattern of T cell-mediated response against the exogenous antigen promoted by the said immunoregulatory material.

8. A method according to claim 7, wherein the immunoregulatory material is as defined in any one of claims 2 to 6.

9. The use in the manufacture of an immunotherapeutic composition for use in treatment of the human or animal body by promoting the T cell-mediated response to an exogenous antigen of immunoregulatory material derived from a mycobacterium other than M. tuberculosis.

10. The use according to claim 9 of immunoregulatory material as defined in any one of claims 2 to 6.

11. A pharmaceutical formulation comprising an association of immunoregulatory material derived from a mycobacterium other than M. tuberculosis and an antigen exogenous to the mycobacterium, and one or more diluents or carriers therefor.

12. A formulation according to claim 11 comprising immunoregulatory material as defined in any one of claims 2 to 6.

International Application No PCT/GB 91/01969

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)*

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 39/39

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,8505034 (UNIVERSITY COLLEGE LONDON) 21 November 1985, see pages 1-3; claims (cited in the application)	1-12
X	WO,A,9007935 (AUSPHARM INT. LTD) 26 July 1990, see the claims	1,8,9, 11
Y		2-7,10, 12
Y	International Journal of Leprosy and other Mycobacterial Diseases, vol. 57, no. 1, March 1989 (Bloomfield, NJ, US) R. Ganapati et al.: "A pilot study of three potential vaccines for leprosy in Bombay", pages 33-37, see the summary	2-7,10, 12
X,P	WO,A,9101751 (UNIVERSITY COLLEGE LONDON) 21 February 1991, see pages 1,2, claims	1-12

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14-02-1992	16. 03. 92
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: Maria Peis

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 7 and 8 ared directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101969 SA 53078

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 10/03/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8505034	21-11-85	AU-B- 588809	28-09-89
		AU-A- 4297685	28-11-85
		EP-A,B 0181364	21-05-86
		JP-T- 61502258	09-10-86
		US-A- 4716038	29-12-87
WO-A- 9007935	26-07-90	AU-A- 4959990	13-08-90
		EP-A- 0454735	06-11-91
WO-A- 9101751	21-02-91	AU-A- 6188390	11-03-91